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EVALUATION OF NITROGEN FERTILIZER AND ROW SPACING EFFECT ON YIELD AND PHYSIOLOGICAL CHARACTERISTICS OF MAIZE (*Zea mays* L.) UNDER IRRIGATED AND RAINFED CONDITIONS

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ABSTRACT

This research aims to investigate the yield and physiological traits of Hybrid 704 single cross maize under rainfed (without any irrigation) and irrigation based on plant needs as split-plot experiment in a randomized complete block design with three replications during 2017-2018. It was carried out in Langrud, Gilan province, Iran. The total amount of precipitation during plant growth period was 580.4 and 463.4 mm in 2017 and 2018 respectively, which happened with improper distribution during the plant growth. In experimental treatments, the first factor includes irrigation and rainfed in the main plots and the second factor was the combination of urea fertilizer levels (control, applying of 100, 200 and 300 kg per hectare) with row spacing (10, 20 and 30 cm distance) were in sub-plots. The results showed that the effect of nitrogen source and row spacing under rainfed and irrigation conditions was significant for all investigated traits in this experiment. In the conditions of rainfed, the maximum grain yield was 11394.6 kg.h^{-1} for applying 300 kg nitrogen fertilizer per hectare and 20 cm row spacing. Under irrigation conditions, the highest grain yield was assigned to 200 and 300 kg of nitrogen fertilizer per hectare and of 20 cm row spacing, with an average of 14050.5 and 14993 kg per hectare, respectively. In addition, an increase in physiological traits, including relative water content, proline, antioxidant activity, and improvement of electrolyte leakage under rainfed conditions was observed by applying nitrogen fertilizer and increasing the row spacing. As a result, in rainfed conditions, using 200 to 300 kg of nitrogen fertilizer and increasing the row spacing should be used to obtain the highest maize yield in the experimental area.

Keywords: Catalase, electrolyte leakage, proline, relative water content.

INTRODUCTION

One of the most popular challenges in the contemporary era is ensuring food security for all humanity all around them. To solve the problem of sustainable food supply as well as protect the environment and natural resources, it is necessary to use solutions that simultaneously increase food production, protect the environment and make optimal use of resources and input (Li et al., 2021). On the other hand, population increase, improper water management, climate changes and global warming have made water resources face major environmental, social, political and economic challenges (Sattar Shahadha et al., 2021). The global water crisis, including in Iran, has increased the need to pay more attention to the cultivation of rain-fed crops. Most agricultural production depends on precipitation, and precipitation-induced water infiltration into the soil and water storage in the crop's rhizosphere is an important part of Rainfall Efficiency Index. Therefore, considering the problems related to the water sector in Iran and capacity of agricultural production based rainfall in this country, increase in Rainfall Efficiency Index is an effective way to achieve food security (Mirjalili et al., 2022).

Maize is one of the most productive cereals belonging to the family *Poaceae* and tribe *Maydeae* after wheat and rice. The use of different parts of maize as feed for livestock and poultry plays an important role in the production of protein products. Thus, the proportion of maize for human consumption is 20-25%, livestock and poultry feeds is 60-75%, and raw materials for industrial products is 5% (Álvarez-Iglesias et al., 2021). Maize is widely cultivated in many countries due to its ability to adapt to various climatic conditions, relative resistance to drought, and high yield. The area under cultivation of maize has increased in most countries of the world in recent years, due to adaptation of maize to various weather conditions, increasing demand for industrial products and animal feed, consequently, maize has gradually become one of the most popular cereals in most parts of the world (Yang et al., 2022).

Among fertilizers, nitrogen (N) is important because it is responsible for the main activities for the growth (Moshki et al., 2017) and development of maize. The amount of nitrogen a plant needs depends on the initial amount of nitrogen, the texture and moisture of the soil (amount and distribution of precipitation), the amount and duration of irrigation, and the type of cultivation. After water stress, nitrogen is the second limiting factor for seed growth in arid regions (Nouri et al., 2020). Nitrogen deficiency increases the effects of water stress on crops and on the other hand, nitrogen fertilizer management in rainy conditions is difficult due to unpredictable soil moisture and its effect on growth of plants (Pandit et al., 2022). Therefore, the response to fertilizer use management will vary from place to place in terms of quantity and timing of fertilizer application (Ozturk and Yildirim, 2013). After awareness of the need for sufficient amount of nitrogen fertilizers to achieve optimal production efficiency of plants, awareness about the appropriate time to use them will increase the efficiency of use and the optimum yield of these crops (Malesevic et al., 2010). Nitrogen management and improper irrigation are considered to be the main factors that reduce maize yield. Under soil conditions where water deficiency affects the absorption of nutrients, especially nitrogen, there is a need to establish a balance between nitrogen consumption and soil moisture availability (Yang et al., 2022).

One of the important factors related to crops is choosing appropriate plant density per unit area. Proper density and balanced distribution of plants per unit lead to better use of moisture, nutrients, light, and finally, yield of crops is increased. The yield of each plant is the result of competition between them to acquire growth structures. The highest plant production per unit area is achieved when these competitors reach the lowest value and the plant can utilize natural resources approvingly (Zhang et al., 2020). Low plant density can drain soil moisture during the early growing season and expose plants to drought during reproductive growth. Therefore, it is very important to use different agricultural management practices in rainfed conditions, as well as the amount and timing of fertilizers used in these areas (Zhang et al., 2022).

High crops yield is achieved only when the number of plants per unit area is at an optimal level (Choudhury et al., 2022). It has been reported by Sherestha et al. (2018) that in maize, at low plant density, although the most of the dry matter is allocated to the cob, increasing the row spacing decreases the amount of dry matter allocated to the cob is compensated by the increase in the number of cobs per unit area, So, the grain yield per unit is increased. However, the increase in the number of plants and the lack of water or nutrients in the soil, the desired result may not be obtained.

Therefore, understanding the optimum nitrogen rate can help plan for sustainable production in each region, and understanding its capabilities and limitations is paramount. Gilan Province, due to its suitable ecological conditions and abundant rainfall, is capable of rainfed crops in other parts of Iran. The objectives of this study were to investigate the effects of nitrogen fertilizer and plant density under rainfed and irrigation conditions on physiological characteristics and yield of maize.

MATERIALS AND METHODS

Study site

Samples were collected from field soil of Langrud in Gilan province, Iran (Long. 48˚ 29´ E, Lat. 36˚ 27´, 14 m a.s.l. Fig.1) during 2017-2018. The climate of the area is temperate, which is the result of the impact of the Alborz Mountain chain and Caspian Sea. The relative humidity of the province was between 40 and 100 percent due to its proximity to the Caspian Sea. The drought season does not last during the year (about one month from half of July to mid-July) and in the most rainy days during the year.

Prior to the experiment, the physicochemical properties of the surface soil were determined and the results are shown in Table 1. The climatic properties of the plant growth period are shown in Table 2.

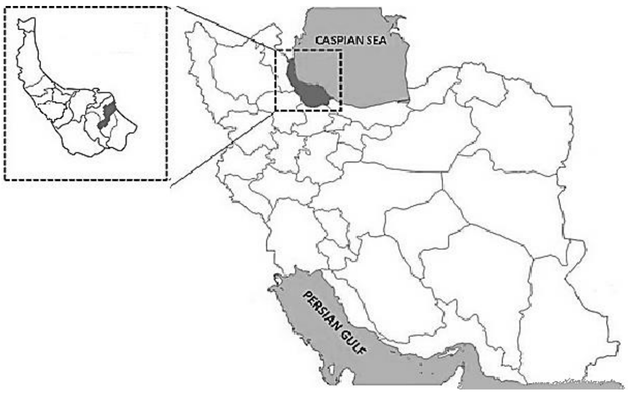

Figure 1. Location of studied region and wheat fields

Table 1. Physical-chemical properties of soil

Sampling depth (cm)	pH	OC (%)	EC (ds.m-1)	N (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	6	1.7	0.17	0.14	7.4	138	43	21	36	Sandy clay
30-60	6.2	0.8	0.17	0.08	3.4	99	43	21	36	Sandy clay

Table 1. Weather data of the experimental site during the maize (*Zea mays* L.) growing period at Langrud, Gilan, Iran during 2017 and 2018.

2017	Min.Temp (°C)	Max.Temp (°C)	Rainfall (mm)
March	5.3	14	117.1
April	4.4	14.2	148.7
May	7.9	17.6	133.4
June	12.3	21.6	44.6
July	17.4	27.3	19.1
August	20.2	30.4	117.5
Mean	11.25	20.85	96.7
2018			
March	3.1	11.7	81.4
April	0	10.3	190.2
May	7.2	14.9	139.4
June	11.6	21.1	24.4
July	17.3	27.3	7.3
August	20.4	29.8	20.7
Mean	9.9	19.1	77.2

Sampling and laboratory analysis

Split-plot randomized complete block designs (RCBD) with three replications were performed. Experimental treatments include different levels of urea (control, application of 100, 200 and 300 kg per hectare), the time and amount of nitrogen fertilizer are presented as three times: one-third at the time of planting, one-third at 7-8 leaf and the last in the pollination phase. Different row spacing included distance of plant on row 10, 20 and the 30 cm under irrigation and rainfed conditions. The surface irrigation took place (furrow irrigation method). Plot size was 16 square meters (8 × 2 square meters) and in each plot distance between rows 75 cm, plant spacing on row based on expressed treatments, distance between second plots was 50 cm and the distance between main plots was two meters. For full irrigation conditions, the irrigation interval was six days. The amount of water used in two years of experiment, 330 and 360 ml was recorded.

The amount of fertilizer used based on the soil experiment results (Table 1) included 200 kg per hectare of superphosphate and 150 kg per hectare of potassium sulfate, which were used during tillage. The cultivar used in this experiment was Single Cross 704, which was planted on June 1, 2017- 2018. The seeds were first disinfected with Vitavax (with a ratio of two per thousand). and planting depth was five centimeters. Avant and Consult poisons were used during plant growth against pests. Weed removal was done manually during the growth stage.

To calculate the biological yield, after removing the marginal effect, 15 plants were randomly selected and harvested. Then, the plants were placed in an oven at a temperature of 70 °C for 48 hours. After that dry weight was measured using an accurate scale. The harvest index was obtained from the ratio of grain yield to biological yield.

Total nitrogen was measured by titration after distillation using an automatic Kjeldahl analyzer, and after measuring nitrogen percentage, grain protein percentage was obtained by multiplying by 6.25. The economic nitrogen use efficiency (grain yield/grain protein) and economic water use efficiency - WUE (grain yield/ water consumption) were also calculated.

The relative water content was determined ((fresh weight of leaves - dry weight of leaves)/ (weight of leaves in the turgor - dry weight of leaves) × 100) according to the method of Askari and Ehsanzadeh (2015).

The amount of proline in leaves was measured using the method of Bates et al. (1973), and the percentage of electrolyte leakage was measured ((Electrical conductivity measurement after 24 hours/ Electrical conductivity measurement after 20 minutes of autoclaving at 120 °C) × 100) according to the method of Sullivan and Ross (1979).

The activities of catalase and peroxidase enzymes were also analyzed by Aebi's method (1984).

Statistical analysis

To test normality and equality of variances, Kolmogorov-Smirnov and Barlet's tests were employed, respectively. The difference between parameters was tested by combined analysis of variance followed by LSD test. All statistical analyses were performed using SAS software (9.3) (SAS Institute, 1997), to examine associations between properties a multivariate Pearson correlation analysis based on Principal Components Analyses Ranking (PCA Ranking) was performed using R version 4.3.19 (McCune and Mefford, 1999).

RESULTS AND DISCUSSIONS

In this study, the effects of years on harvest index, grain protein content, proline content, relative leaf water, catalase activity and economic water use efficiency had a significant difference ($p<0.01$) and the nitrogen use efficiency and the amount of electrolyte leakage were significantly affected by years ($p<0.05$). The effect of irrigation type on all factors except grain yield and harvest index had the effect significant, and nitrogen fertilizer on all traits was statistically significant. In this study, the effect of row spacing was also statistically significant ($p<0.01$) on all traits except electrolyte leakage (Table 3).

The interaction between irrigation and nitrogen fertilizer had a significant effect on all traits except harvest index, electrolyte leakage, and catalase activity. The interaction of irrigation with row spacing also showed a significant effect ($p<0.01$) on biological yield, grain protein, electrolyte leakage, peroxidase activity and economic water use efficiency. The interaction of nitrogen fertilizer and row spacing was also significant on biological yield, grain protein, nitrogen use efficiency, proline content, catalase and peroxidase activity, and economic water use efficiency at the level of 1% (Table 3).

Table 3. Analysis of variance of the effect of irrigation. Nitrogen level and plant density on functional and physiological traits of maize (*Zea mays L.*) During 2017-2018

S.O.V	d.f	S.Y	B.Y	H.I	PRO.	NUE	Prolin	RWC	E.C	CAT	Proxi	WUE
Year	1	1205^{ns}	765042^{ns}	939.6^{**}	4^{**}	372.4^{*}	0.00003^{**}	202.7^{**}	34^{*}	0.3^{**}	35.3^{ns}	3^{**}
R(Year)	2	1	1384	2.2	0.01	4	0.0000009	1.5	0.23	0.06	0.12	0.03
A	1	114.6^{ns}	10962721^{**}	35^{ns}	17^{**}	1385^{**}	0.002^{**}	8808^{**}	4264^{**}	26.7^{**}	31385^{**}	0.94^{**}
A*Year	2	0.03^{ns}	12882^{ns}	0.31^{ns}	0.01^{ns}	0.004^{ns}	0.0000001^{ns}	1^{ns}	0.006^{ns}	0.03^{ns}	0.003^{ns}	0.005^{ns}
Error a	1	0.01	8182	0.61	0.004	0.2	0.0000009	3	0.003	0.005	0.13	0.01
B	3	2067.7^{*}	16349358^{**}	303.2^{*}	111.7^{**}	28571.3^{**}	0.006^{**}	348.8^{**}	34.8^{**}	8.7^{**}	5036.8^{**}	1.7^{**}
A*B	3	3282^{**}	1041889^{**}	167.2^{ns}	1.8^{**}	361.7^{**}	0.00009^{**}	108.8^{**}	7.5^{ns}	0.12^{ns}	584^{**}	0.1^{**}
Error B	6	2873.8	683938	110	0.36	89.8	0.0000005	138.4	71.3	1	44	0.05
Year*B	3	0.41^{ns}	19272^{ns}	3.3^{ns}	0.22^{ns}	6.7^{ns}	0.0000003^{ns}	0.34^{ns}	0.05^{ns}	0.03^{ns}	0.09^{ns}	0.16^{**}
Year*A*B	3	0.5^{ns}	34812^{ns}	1.4^{ns}	0.01^{ns}	2.6^{ns}	0.0000003^{ns}	0.71^{ns}	0.03^{ns}	0.05^{ns}	0.27^{ns}	0.009^{ns}
C	2	6752.4^{**}	629273323^{**}	1157.7^{**}	30.3^{**}	2966.3^{**}	0.0003^{**}	446^{**}	16.2^{ns}	22.5^{**}	9817.8^{**}	0.35^{**}
Year*C	2	9^{ns}	26865^{ns}	26.4^{ns}	0.01^{ns}	1.3^{ns}	0.0000009^{ns}	0.94^{ns}	0.13^{ns}	0.008^{ns}	0.001^{ns}	0.01^{ns}
A*C	2	28.7^{ns}	17038555^{**}	247^{nns}	4^{**}	131.2^{ns}	0.0000001^{ns}	20.5^{ns}	354.4^{**}	0.12^{ns}	1152.2^{**}	0.41^{**}
B*C	6	1436^{ns}	2318682^{**}	166.3^{ns}	4.3^{**}	399.2^{**}	0.0003^{**}	10.3^{ns}	0.9^{ns}	0.22^{**}	90.3^{**}	0.21^{**}
Error C	12	1490.4	789612	167.8	26.7	194.8	0.0000001	150.6	35.2	0.6	119	0.05
Year*A*C	2	0.01^{ns}	7106^{ns}	0.75^{ns}	0.02^{ns}	0.7^{ns}	0.0000009^{ns}	0.63^{ns}	0.02^{ns}	0.01^{ns}	0.24^{ns}	0.006^{ns}
Year*B*C	6	0.1^{ns}	4790^{ns}	1^{ns}	0.01^{ns}	2.4^{ns}	0.0000005^{ns}	0.67^{ns}	0.03^{ns}	0.04^{ns}	0.13^{ns}	0.006^{ns}
A*B*C	6	3183^{**}	481346^{ns}	273^{*}	4.6^{**}	126.8^{ns}	0.00002^{**}	4.5^{ns}	11^{ns}	0.15^{ns}	29.2^{ns}	0.32^{**}
Year* A*B*C	6	0.47^{ns}	5698^{ns}	2^{ns}	0.02^{ns}	2^{ns}	0.0000005^{ns}	0.92^{ns}	0.02^{ns}	0.04^{ns}	0.05^{ns}	0.009^{ns}
Total Error	56	681.4	229032	95.6	0.12	59.6	0.0000006	9	7.3	0.06	13.6	0.02
CV (%)	-	20	4.6	15.5	5.8	13	3	3.6	12.4	5.4	2.7	11.5

ns, * and **: insignificant and significant at 1 and 5% probability levels, respectively. A: Irrigation, B: Nitrogen level, C: Plant density, S.Y: Seed Yield, B.Y: Bilogical Yield, H.I: Harvest Index, Pro. Seed Protein, NUE: Nitrogen Use Efficiency, RWC: Relative Water Content, E.C: electrolyte leakage, CAT: catalase, Proxi: peroxidase, WUE: Water Use Efficiency.

According to the mean comparison results, grain yield increased from control to 100, 200 and 300 kg/ha under irrigation and rained methods with increasing nitrogen fertilization. Therefore, for the irrigation method, the highest yield of 10689.3 kg per hectare was recorded with applying 200 kg, which was not significantly different from 300 $kg.ha^{-1}$. Under rainfed method, the highest grain yields, 11394.6 kg per hectare, achieved in 300 $kg.h^{-1}$. Moreover, under irrigation and the rainfed methods the highest grain yield were at row spacing of 20 and 30 cm. finally, the lowest value was associated with a spacing of 10 cm .

Higher row spacing increases competition among plants for light, water, and food, thus reducing grain weight per plant and plant height. Increasing the number of plants per unit area affects or increases biological yield more than cereal yield. Therefore, at higher densities, the harvest index is lower than its average density.

In this study area, the highest maize grain yield per hectare was achieved in rainfed conditions with the application 200-300 kg of nitrogen fertilizer per hectare and at row spacing of 20 cm. A key factor in high yields is application of nitrogen fertilizers at the right time and in the right amount during the growth and development stages of crops in the Mediterranean Region. However, plant responses to nitrogen fertilizer application positively correlated with plant variety, irrigation management, rainfall and distribution during its life cycle, and local climatic conditions (Malesevic et al., 2010). Ercoli et al. (2008) showed that with increasing drought stress levels in post-flowering of durum wheat, seed weight, and number of seeds per panicle, harvest index, and grain yield decreased significantly at different levels of nitrogen fertilizer. However, the reduction of these traits increased with increasing nitrogen fertilizer content. Moreover, Nasiri et al. (2008) reported that nitrogen fertilizer had no effect on the severity of post-flowering drought stress in wheat and significantly increased grain yield with application of 80 kg of nitrogen per hectare under full and low irrigation. Therefore, due to rainfall and high humidity in this study area, it is possible to apply 200-300 kg of nitrogen per hectare using the split method under rainfed conditions and achieve grain yield similar to that obtained under irrigation. The results of this study showed that the number of plants per unit area directly affects grain yield and that increasing spacing to a certain level (row spacing of 20 cm) may lead to increased grain yield. Under the irrigation condition with row spacing of 20 cm, the yield increased by 91% compared with 10 cm, 1.3 times higher than that under the rainfed condition.

A study by Zhai et al. (2022) found that consuming higher amounts of nitrogen led to plant growth when water was not the limiting factor for plant growth. Besides nitrogen element promotes vegetative growth of plants which result in the amount of evaporation and transpiration and thus removing water from the soil. This resulted in reduction in amount of dry matter produced per unit of nitrogen consumption and reduction in nitrogen used efficiency. The increased grain protein and nitrogen used efficiency in this study may be associated with increased precipitation and the presence of high humidity in Gilan, resulting in reduced adverse effects of water stress on plants.

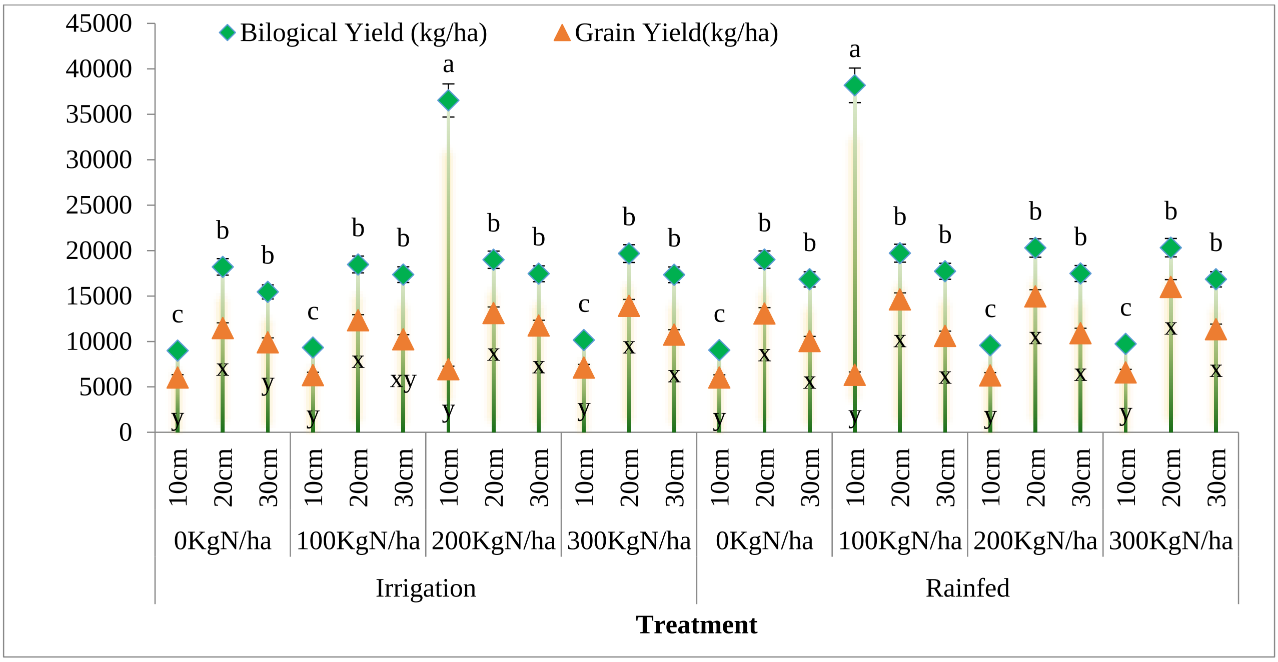

Figure 2. Mean comparison of some yield traits under the effects of different irrigation, planting density and N fertilizer on maize (*Zea mays* L.)

Results showed that under irrigation conditions with no fertilization and application of 100 kg per hectare nitrogen, harvest index increased with decreasing row spacing (10 cm spacing). With application of 200 kg and 300 kg nitrogen per hectare, the highest HI was associated with a space of 20 cm. The highest harvest index was therefore recorded under irrigation with application of 300 kg per hectare at distance of 20 cm (68.5%). The highest of HI for all fertilizer levels was associated with a planting distance of 20 cm under rainfed condition. The maximum amount of HI, 76.5%, was observed at a row distance of 20 cm with the application of 300 kg nitrogen per hectare.

The results of this study showed that electrolyte leakage in rainfed was higher than in irrigation conditions and its amount of rainfed was greater than in all cultivation intervals than full plant irrigation.

Electrolyte leakage was significantly increased in rainfed conditions compared to irrigation condition. For example, rainfed had 66% higher electrolyte leakage than irrigation condition. Moreover, in both irrigation and rainfed conditions, electrolyte leakage decreased with increasing spacing between plant rows. Under rained condition the amount of leakage was higher at all row spacing. The absence of fertilizer and at row spacing of 10 cm were shown the highest leakage with an average of 23.4%, and electrolyte leakage decreased with increasing in nitrogen fertilizer and row spacing. One of the first parts of a plant to be damaged under water stress is the plasma membrane. This is because, under stress conditions, the generation and accumulation of reactive oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals increase. These compounds damage lipid, proteins, carbohydrates, and nucleic acids and alter structure of membranes by oxidizing lipid and proteins, making cell membranes more permeable and allowing electrolytes to leak out of cells (Amer Dahham, 2021).

Nitrogen availability increases in some amino acids under plant stress conditions. Islam et al. (2009) depicted that the application of different amounts of nitrogen fertilizer under cold stress, decreased electrolyte leakage with increasing amounts of nitrogen used, and the highest leakage was observed in the control treatment. Studies have reported improvements in electrolyte leakage under stress conditions with application of nitrogen fertilizers (Shafe et al., 2011; Akhtari et al., 2014). Increased nitrogen consumption through increased protein synthesis, increased cell wall thickness, increased water uptake by the cytoplasm, improved relative water content of leaves, and reduces electrolyte leakage (Namvar and Khandan, 2015).

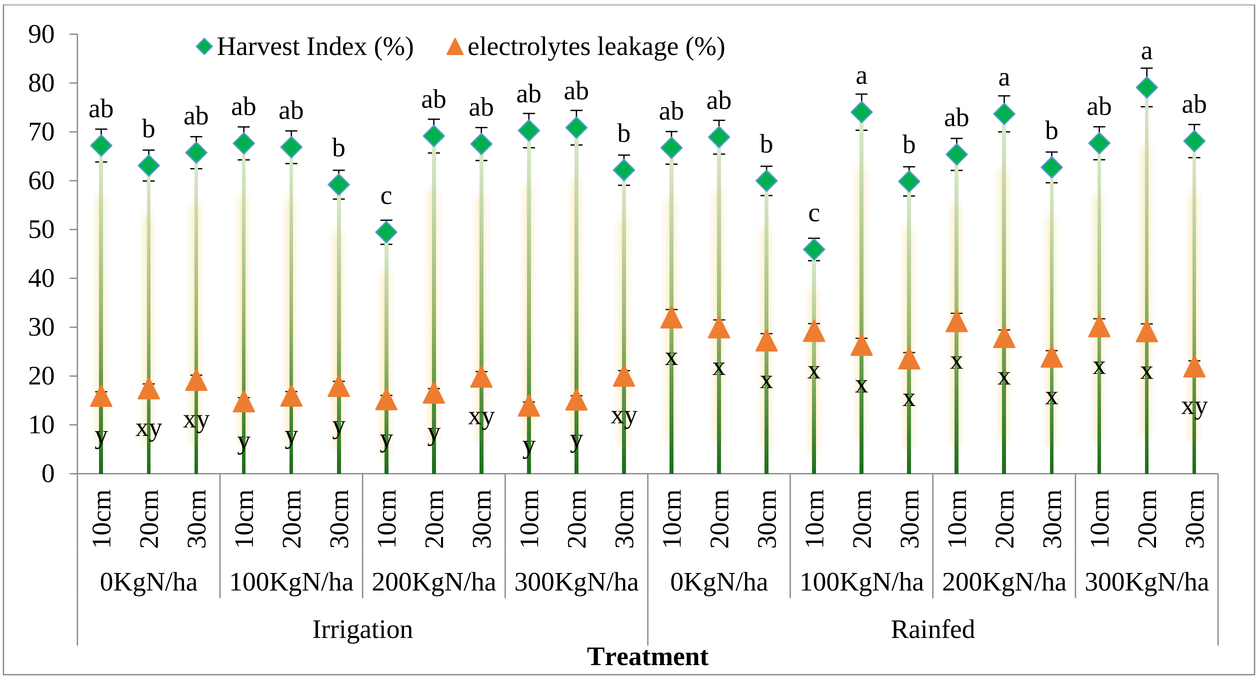

Figure 3. Mean comparison of some harvest index and electrolyte leakage under the effects of different irrigation, planting density and N fertilizer on maize (*Zea mays* L.)

The highest water use efficiency (WUE), averaging 2.06 kg/m-3, was observed when the plants were fully irrigated and 100 kg/ha nitrogen fertilizer was used for row spacing of 30 cm. Under rainfed and no fertilization resulted in the lowest WUE, averaging 0.78 kg/m-3 at a space of 20 cm. In this study, using nitrogen fertilizer under rainfed and irrigation conditions improved WUE. Nevertheless, the effect of nitrogen fertilization on WUE under full irrigation method was greater than that under rainfed condition at all row spacing. Moreover, the highest WUE of 1.63 kg/m^{-3}, was observed with 300 kg of fertilizer and row spacing at 10 cm (Figure 2-4). Due to the high rainfall and humidity in Gilan Province, the leaching of nitrogen from the soil is also increasing. Therefore, in rainfed conditions in this research, an increase in nitrogen consumption resulted in water use efficiency. As a result, in both irrigation methods with nitrogen application, the water use efficiency at row spacing of 30 cm was higher than the other 10-20cm. At higher spacing rows, there is no adverse impact from competition between plants resulting in more water use efficient, whereas at fewer spacing rows creates stressful conditions during the reproductive growth stage and yield reduces in rainfed more than the irrigation condition, which will result in increased water efficiency. In present study a row spacing of 30 cm led to increased grain yield and plant biomass, thereby increased in economy water use efficiency at the specified row spacing.

Leaf proline content increased in rainfed conditions compared with irrigation conditions. For both irrigation methods, leaf proline content increased with increasing urea fertilizer application and increasing row spacing. Therefore, the highest amount of 0.037 mg/g fresh leaf weight was observed at 300 kg/ha nitrogen and a row space of 30 cm under rainfed condition. Among the various cellular mechanisms that occur during stress, proline accumulation is particularly important. Proline is a neutral amino acid which highly water-soluble, and may account for up to 80% of amino acids in cells under stress (Amer Dahham, 2021). When plants are stressed, the accumulation of intracellular proline increases to absorb and retain water. Proline is known as a messenger and regulatory molecule for a variety of responses, and a significant increase in proline under stress conditions was aimed at maintaining cell survival (Altuntas et al., 2019). Most of the proline composition has a nitrogen structure. Therefore, using nitrogen can greatly increase the amount of nitrogen in the plant. In addition, chemical nitrogen fertilizers are readily available to plants due to the accessibility and dynamics of the nitrogen element.

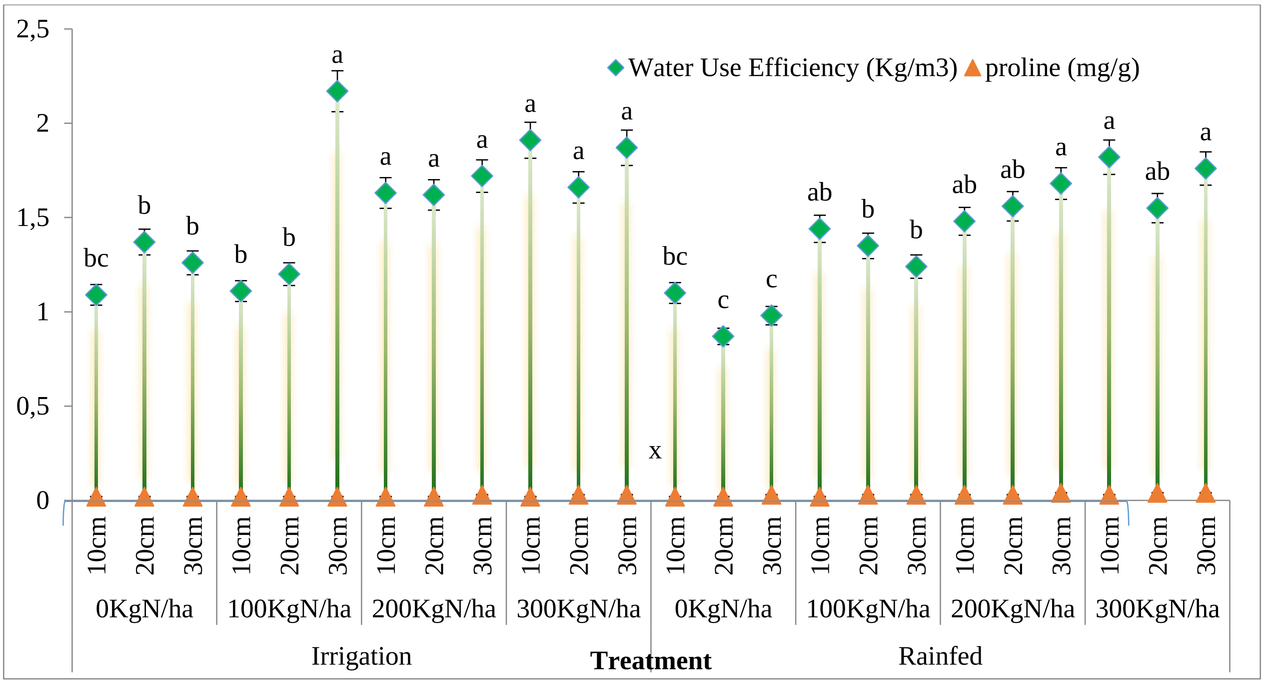

Figure 4. Mean comparison of some water use efficiency and proline under effects of different irrigation, planting density and N fertilizer on maize (*Zea mays* L.)

A comparison of catalase mean values also showed an increase in nitrogen fertilization at all planting densities. The enzyme activity was lowest at a fertilization rate and planting distance of 10 cm, and the enzyme activity increased with increasing nitrogen fertilization and planting distance. Furthermore, the activity of this enzyme increased significantly under rainfed conditions compared with watering the plants.

Both full irrigation and rainfed with nitrogen fertilizer increased the amount of seed protein. In addition, increasing the planting distance from 10 cm to 20 cm and 30 cm also increased the percentage of seed protein. Therefore, treatment with 300 kg/ha nitrogen fertilizer and 30 cm planting distance resulted in the highest amount of protein, averaging 9%, with no significant difference between the two irrigation methods. The dry method, no fertilization, and a planting distance of 10 cm showed the lowest value of 2% on an average.

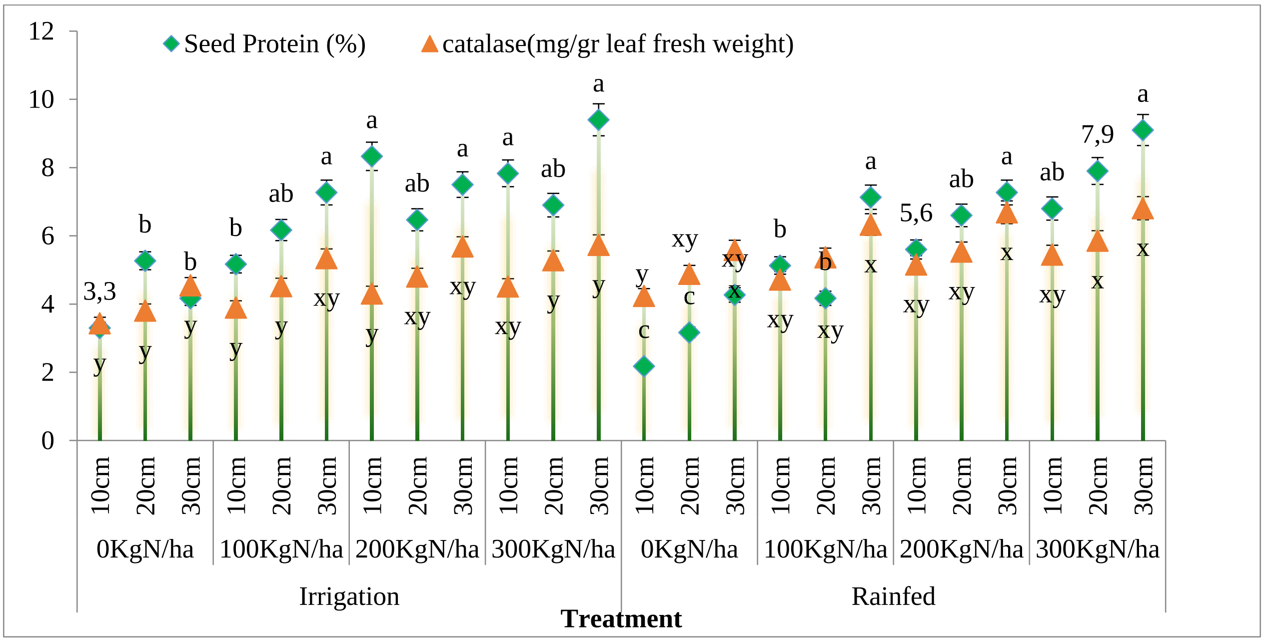

Figure 5. Mean comparison of some seed protein and catalase under the effects of different irrigation, planting density and N fertilizer on maize (*Zea mays* L.)

The efficiency of nitrogen utilization in both irrigation methods was increased by using nitrogen fertilizers. The fertilization of 100 kg/ha and full irrigation and upland farming greatly improved nitrogen consumption efficiency. Application of 200 kg/ha and 300 kg/ha of nitrogen fertilizer reduced irrigation by 10 and 30% and 11 and 16.6%, respectively, in dry cultivation compared with the application of 100 kg/ha of fertilizer. In both irrigation methods, the longer the planting distance, the higher the nitrogen consumption efficiency. The highest values of 71.2 or 65.7 kg/kg on average were measured using full irrigation or dry cultivation at a planting distance of 30 cm. The results also showed that nitrogen consumption efficiency with fertilizer increased from no consumption to 100, 200 and 300 kg/ha at all planting intervals. A maximum of 96.5 kg/kg was observed with 100 kg/ha of fertilizer applied and planted at 30 cm intervals.

All levels of nitrogen fertilizer increased the relative content of water in leaves under irrigation compared with rainfed plants. There was no statistically significant difference between 100 and 200 kg/ha of nitrogen fertilizer. The relative moisture content for dry farming averaged 78% without fertilizer application, which was somewhat higher than averages of 77 or 74.2% with 100 or 200 kg of fertilizer applied per hectare. The application of 300 kg of fertilizer reduced the relative water content (RWC) by only 4% and 14% for irrigated and dry cultivation methods, respectively, compared to no fertilizer application. The slight decrease in RWC when treated with 300 kg nitrogen is likely due to increased leaf area and transpiration.

A comparison of the average values the same catalase also showed an increase in peroxidase enzyme activity due to nitrogen fertilization at all planting densities. Enzyme activity was lowest at fertilization amount and planting distance of 10 cm, and enzyme activity increased with increasing nitrogen fertilization and planting distance. Also, the activity of peroxidase enzyme was significantly increased in rainfed conditions compared to watering plants.

Plants under rainfed conditions minimize intercellular spaces and the amount of water in the organs by increasing osmotic material in the tissues, so that water from the soil enters them with a greater force, which seems to lead to a decrease in RWC (Shukla and Panda, 2023). Conflicting results have been reported regarding the effect of nitrogen application on relative water content. Some researchers have found that the use of nitrogen fertilizers reduces the leaf water content per unit of leaf area due to its reduced content (Aliarab et al., 2020). Namvar et al. (2013), in a study on chickpea plants, suggested that plants fed more nitrogen fertilizers had higher leaf relative humidity, attributed to the greater ability of these plants to sustain potential pressure on leaves.

Based on the results, the activities of catalase and peroxidase enzymes were significantly increased under rainfed conditions compared with the irrigation method. Studies have shown that under environmental stress conditions such as drought stress, the presence of free radicals increases the concentration of antioxidant enzymes in the leaf tissue. Increasing in activity of the catalase and peroxidase enzymes results in the loss of hydrogen peroxide produced in cells under stress, thus limiting cell damage and increasing the plant's oxidative capacity to cope with drought stress (Liu et al., 2008). Zafari et al. (2020), in a study conducted on the biochemical properties of plants under drought stress, stated that the activities of antioxidant enzymes such as catalase, superoxide dismutase, and proline were increased under drought stress.

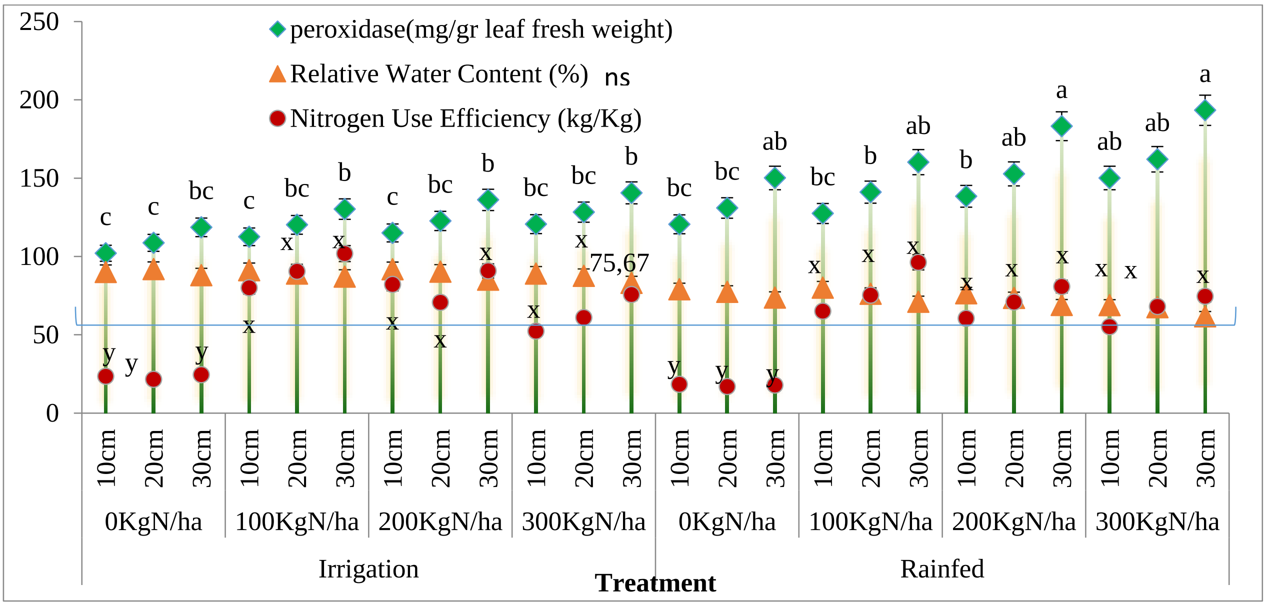

Figure 6. Mean comparison of peroxidase, relative water content and nitrogen use efficiency under effects of different irrigation, planting density and N fertilizer on maize (*Zea mays* L.)

The correlation between the studied traits showed a strong positive correlation among the amount of peroxidase with prolin and catalase content, and a negative correlation between biological yield and harvest index (Figure 7). Interaction results between the irrigation method and row spacing showed that the enzyme activity increased in all three row spacing used in rainfed conditions compared with full irrigation. In addition, the row spacing of 30 cm showed the highest enzyme activity for both irrigation methods. When plants are exposed to stressful conditions, oxidative stress occurs, free radical attack the increased the peroxidation of unsaturated fatty acids and lipids. A correlation between an increased proline content and antioxidant enzyme activity and decreased electrolyte leakage in plants has been reported (Liu et al., 2008). In this study, improvement in electrolyte leakage under stress conditions was observed at row spacing of 30 cm. This may be due to increased antioxidant enzyme activity. Movludi (2015) also reported that increased nitrogen application increased catalase enzyme production in spring barley. Application of nitrogen fertilizers in the present study resulted in a decrease in electrolyte leakage, and increasing activity of antioxidant enzymes, including catalase, increased the proportion of chloroplasts and increased the production of reactive oxygen species by preventing damage. It inhibits biomolecules, including membrane lipids, and prevents electrolyte leakage (Franklin et al., 2010).

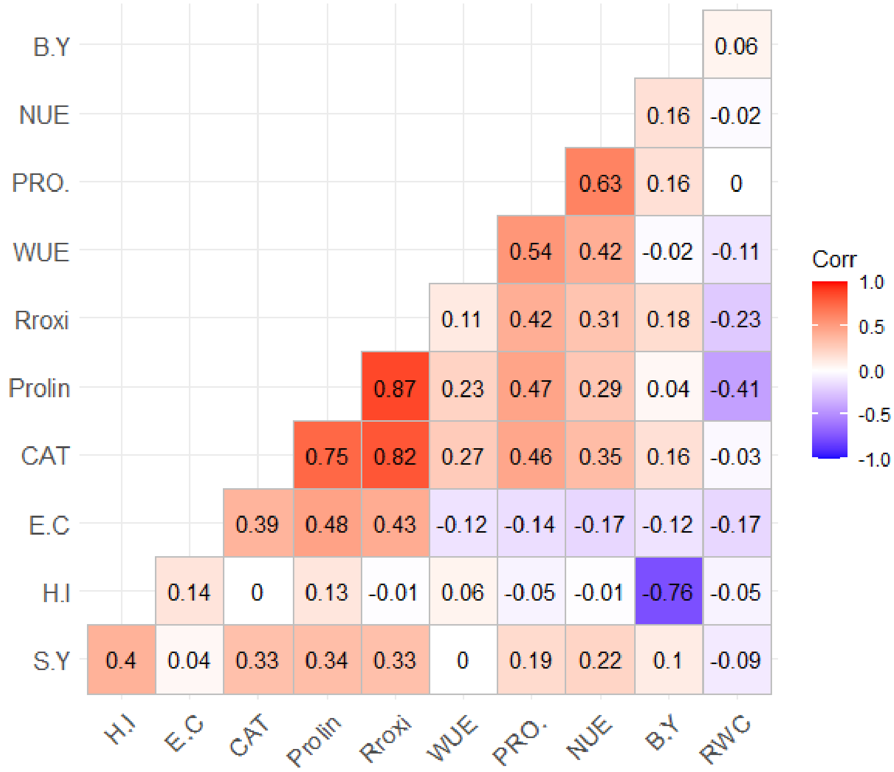

Figure 7. Correlation plot of traits of maize (*Zea mays* L.) under different treatments. S.Y: Seed Yield, B.Y: Bilogical Yield, H.I: Harvest Index, Pro.: Seed Protein, NUE: Nitrogen Use Efficiency, RWC: Relative Water Content, E.C: electrolyte leakage, CAT: catalase, Proxi: peroxidase, WUE: Water Use Efficiency.

In order to more accurately assess the relationships between the characteristics of Maize under different treatments, a major component analysis was conducted (Figure 8). As the graph shows, the first and second components represented about 40.2% and 18.5% for the first year and 39.3% and 23.2% for the second year, respectively. The cosine of the angle between two vectors estimates the correlation between them; therefore, clustered points are highly correlated with each other. There are three clusters of variables that are strongly correlated with each. The first and third clusters are highly correlated with catalase, peroxidase, proline, and electrolytes leakage. Running perpendicular to the first cluster, the second cluster of highly correlated variables includes biological yield, relative water content, water use efficiency, seed protein and nitrogen use efficiency (Figure 8). The control treatment in N1 has no correlation with all parameters. However, N2 and N3 have correlation with clusters 2, and N4 with cluster 1 and 3. All plant density levels are highly correlated with the three clusters. Moreover the first and thirth clusters are highly correlated with I2, while the second cluster is highly correlated with I1 (Figure 8).

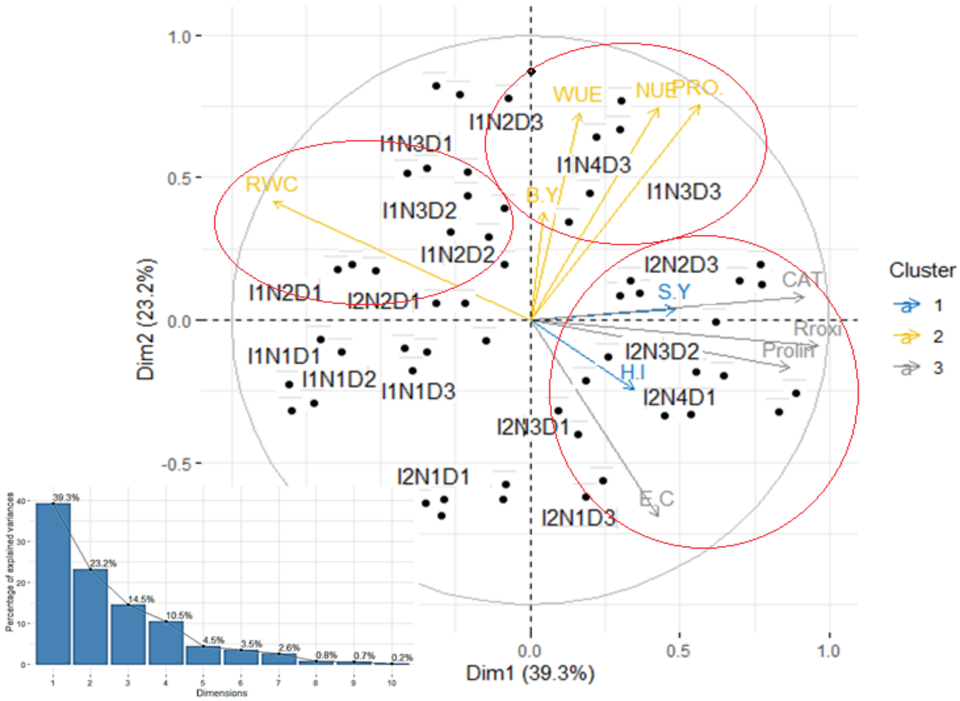

Figure 8. Principal Component Analysis (PCA) of data for all characteristics under different Nitrogen (N1:0,N2:100KgN/ha,N3: 200KgN/ha, N4: 300KgN/ha), Irrigation (I1: irrigated,I2: rainfed) and Plant density (D1:10cm, D2:20cm,D3:30cm) . S.Y: Seed Yield, B.Y: Bilogical Yield, H.I: Harvest Index, Pro.: Seed Protein, NUE: Nitrogen Use Efficiency, RWC: Relative Water Content, E.C: electrolytes leakage, CAT: catalase, Proxi: peroxidase, WUE: Water Use Efficiency.

CONCLUSIONS

The results depicted that physiological traits including relative water content, proline content, antioxidant activity increase while electrolyte leakage decreases under rainfed conditions when nitrogen fertilizer was applied and row spacing was increased.

Also, it can be seen the highest maize yield in the experimental area under rainfed conditions, the application of 200 to 300 kg/ha of nitrogen fertilizer, and increasing the row spacing to 20 cm should be recommended. Moreover, nitrogen availability led to an increase in grain protein content and improved nitrogen use efficiency. The high rainfall and humidity in the study area contributed to reduced water stress and increased water use efficiency, particularly at a row spacing of 30 cm. Additionally, under rainfed conditions, electrolyte leakage was higher compared to irrigation, but applying nitrogen fertilizer and increasing antioxidant enzyme activity reduced electrolyte leakage.

Overall, the findings suggest that optimizing nitrogen fertilizer levels and row spacing can significantly impact maize yield and physiological characteristics, especially under rainfed conditions. These results provide insights for improving agricultural practices and enhancing maize production in similar regions.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or personal relationships.

STATEMENTS AND DECLARATIONS

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